

## REVIEW AND INTERPRETATION

# Ecological importance of *Neotyphodium* spp. grass endophytes in agroecosystems

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## Keywords

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## Abstract

*Neotyphodium* spp. fungal endophytes form symbiotic associations with agronomic grasses with expression ranging from mutualistic to parasitic. In general, endophyte infection frequencies seem more variable in natural compared to pastoral situations, suggesting that expression and benefits attributable to endophyte infection depend on the resource environment in which the host–endophyte complex was formed and the conditions under which it is being grown. Emerging evidence suggests that expression is not always mutualistic or beneficial to the host in terms of productivity and persistence in resource-limited environments. Expression of host–endophyte associations will vary depending on resource availability and environmental conditions that influence host physiology and growth, and herbivore behavior. New research suggest alkaloids and secondary metabolites produced by host–endophyte associations can have physiological functions in addition to acting as herbivore deterrents. Novel host–endophyte associations are being created that maintain insect and disease resistance, while having minimal detrimental impact on mammalian herbivores. The influence of host–endophyte associations on sward composition and soil food webs is only now coming to light. While novel endophyte associations and their attendant mechanisms can be considered beneficial in some respect, the associations may not be as adaptable to stressful or marginal resource environments and could have long-term ecological impacts measured in terms of persistence and total productivity of the sward. We review the extensive published work on host–endophyte interactions and illustrate the complexity of host–endophyte associations and their interactions with environment, and the range of responses that occur.

Many grasses have evolved as symbiotic associations with fungi, including ecto- and endomycorrhizal fungi of the roots (Smith and Read, 1997), and fungi that systemically infect grass shoots. Among the latter, fungi that live their entire life cycle within the aerial portion of the host grass forming nonpathogenic, systemic, and usually intercellular associations are commonly defined as grass endophytes (Bacon and De Battista, 1991). These endophytes are classified in the tribe Balansieae of the family Clavicipitaceae (Ascomycetes) (Diehl, 1950). Within the past two decades, seven genera of grass endophytes were identified, including *Atkinsonella*, *Balansia*, *Balansiopsis*, *Echinodothis*, *Epichloë*, *Myriogenospora*

(White, 1994), and *Parepichloë* (White and Reddy, 1998). The best known grass endophytes are *Neotyphodium* spp. endophytes (e-endophytes), which are an anamorphic stage of *Epichloë* spp., endophytes: *N. coenophialum* (Morgan-Jones & Gams) Glenn, Bacon & Hanlin, found in tall fescue (*Lolium arundinaceum* [Schreb.] SJ Darbyshire, formerly *Festuca arundinacea* Schreb.), *N. uncinatum* (Gams, Petrini & Schmidt) Glenn, Bacon & Hanlin, colonizing meadow fescue (*L. pratense* [Huds.] SJ Darbyshire, formerly *F. pratensis* [Huds.]), and *N. lolii* (Latch, Christensen & Samuels) Glenn, Bacon & Hanlin, in perennial ryegrass (*L. perenne* L.). Their importance is related to the agronomic impact measured in terms of

**Table 1** Similarities and differences between e-endophytes and p-endophytes, and grass host responses to infection

Property	e-endophytes	p-endophytes	Source
Incidence	Pooideae (numerous)	<i>Festuca</i> , <i>Lolium</i> , <i>Achnatherum</i>	Siegel (1993) An <i>et al.</i> (1993) Hayes and Faeth (2004) Li and Nan (2004)
Mycelium in seed	Dense	Sparse	Siegel <i>et al.</i> (1984) Siegel <i>et al.</i> (1995)
Mycelium in leaf	Intercellular; nonbranched, synchronized growth	Inter- or intracellular, highly branched, unsynchronized growth	Christensen <i>et al.</i> (2002)
Mycelium in roots	No	Yes	Hinton and Bacon (1985) Siegel <i>et al.</i> (1995)
Sporulation on host/Infectivity	No	Yes	An <i>et al.</i> (1993)
Host tolerance to biotic and abiotic factors	Yes	?	Schmidt (1993); Malinowski <i>et al.</i> (1997a)
Production of alkaloids	Yes	?	Richardson (2000) Siegel <i>et al.</i> (1995)
Seed dissemination	Yes	Yes	Clay and Schardl (2002)

livestock performance (Oliver, 2005) and sward persistence. Detailed studies with grasses led to the discovery of at least three other groups of symptomless fungal endophytes. One group (p-endophytes) consists of closely related *Gliocladium*-like endophytes (An *et al.*, 1993) in perennial ryegrass (Latch *et al.*, 1984; Philipson, 1991) and *Phialophora*-like endophytes in meadow fescue, *F. gigantea* (L.) Vill., and Arizona fescue (*F. arizonica* Vasey) (Schmidt, 1991; An *et al.*, 1993). The p-endophytes are ordered to Eurotiales (Ascomycetes) (Siegel *et al.*, 1995) and are not related to e-endophytes (Leuchtmann, 1992). There are substantial differences between e-endophytes and p-endophytes not only in their morphology and physiology, but also in grass host responses to infection (Table 1). The third group of grass endophytes (a-endophytes) has been proposed to accommodate endophytes found in Italian ryegrass (*L. multiflorum* Lam.) and *F. paniculata* L. (Naffaa *et al.*, 1998), and other annual species of the *Lolium* genus (Guillaumin *et al.*, 2000). These endophytes are represented by parasitic species of *Acremonium* similar to *A. chilense*, an endophyte of orchardgrass (*Dactylis glomerata* L.) (Morgan-Jones *et al.*, 1990). Similar to p-endophytes, effects of a-endophytes on physiological processes and ecology of cool-season grasses are not well understood at present.

Clay (1988) defined interactions between *Neotyphodium* spp. endophytes and cool-season grasses as defensive mutualism, where infected plants have generally higher resistance to a range of biotic and abiotic stresses (Malinowski and Belesky, 2000). Wilkinson and Schardl (1997) revised the concept and defined these interactions as asymptomatic symbioses. In such associations, the loss of sexuality is a fundamental cost in the long term in contrast to associations that have sexual and asexual means of reproduction. Detailed

studies, however, indicate that symbioses of grasses with *Neotyphodium* spp. endophytes can result both in antagonistic or mutualistic associations, suggesting a continuum from parasitism to mutualism (Saikkonen *et al.*, 1998; Spyreas *et al.*, 2001). It is not known how grass host and the endophyte recognize each other, nor how the grass host interacts at the molecular level to tolerate or eliminate the endophyte. There is circumstantial evidence that grass hosts respond to the presence of *Neotyphodium* spp. endophytes in a way that is similar to infection with pathogenic fungi, at least in part based on secondary metabolites the partners produce (Schulz *et al.*, 1999). These interactions may result in a balanced antagonism and, consequently, coexistence of the symbiota (as in the case of *Neotyphodium* spp. endophytes) or an imbalanced antagonism leading to a disease of the host plant (as for pathogenic fungi) (Table 2).

We outline principal research findings and conclusions on ecological significance of *Neotyphodium* spp. endophytes for agronomic and nonagronomic grasses, and consider the agronomic significance and future application of novel endophytes in agroecosystems.

## Primary driving forces in the evolution of *Neotyphodium*/grass associations

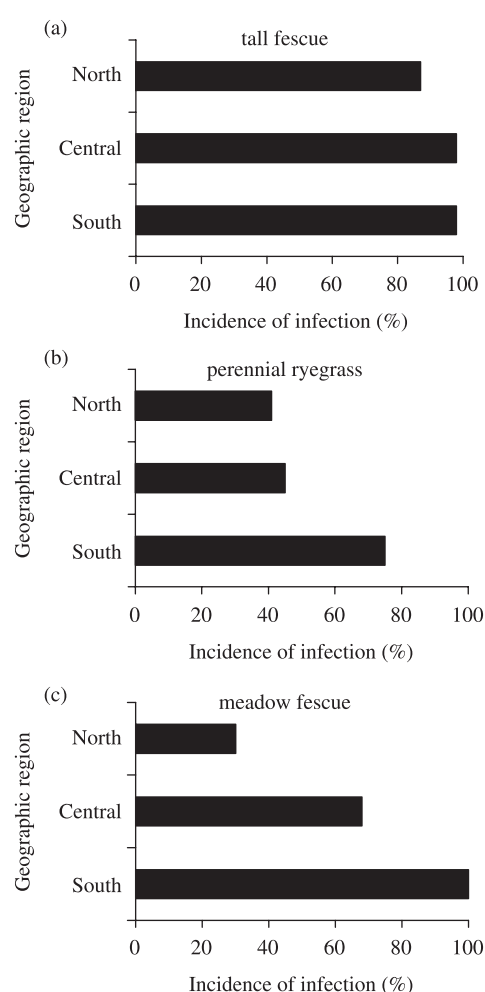
Greater tolerance to biotic stresses, such as protection from herbivores, nematodes, pathogenic fungi, and neighboring plants, could be primary forces in the evolution of *Neotyphodium*/grass associations, resulting in a dominance of endophyte-infected (E+) rather than noninfected (E-) grasses in native grasslands (Schardl and Phillips, 1997). Results obtained on tall fescue and perennial ryegrass pastures in the

**Table 2** Responses of grass hosts to infection with pathogenic fungi and *Neotyphodium*-endophytes

Defense component	Pathogenic fungi	<i>Neotyphodium</i> -endophytes	Source
Chitinase	Yes	Yes	Roberts <i>et al.</i> (1992) Salzer <i>et al.</i> (2001)
Resveratrol	Yes	Yes	Sylvia and Sinclair (1983) Powell <i>et al.</i> (1994)
Other phenolic compounds	Yes	Yes	Malinowski <i>et al.</i> (1998a) Montesinos (2000)
Compatibility	No	Yes/No	Christensen (1995)
Effects on host plants	Imbalanced antagonism resulting in disease	Balanced antagonism resulting in coexistence	

USA and New Zealand seem to support this hypothesis (Popay and Bonos, 2005). Consider, however, that many agronomically important *Festuca* and *Lolium* species evolved in Europe as components of native grasslands, where grazing pressure was modest compared to those encountered in USA or New Zealand. Others propose more complex explanations for evolution of endophyte–grass host associations that involve multi-species interactions, multiple levels of causation and multidirectional flows of influence, which are modified by stochastic events, such as climate, soil and local biota (Saikkonen *et al.*, 2004). Endophyte-infected cool-season grasses occur throughout continental and maritime temperate regions, generally with increased occurrence of endophyte infection detected at lower latitudes. It is quite likely, however, that increased concentrations of a suite of bioactive alkaloids would occur along with the increased infection frequency, because phytoalkaloids increase in numerous plant species as latitude decreases, from cool-temperate to tropical regions (Levin and York, 1978).

The incidence of E+ grasses in Europe may be correlated with climatic conditions (Lewis, 2000b), such that higher endophyte infection rate occurs in drier and hotter regions of southern Europe and lower endophyte infection rates in cool and moist environments of northern Europe (Figure 1). Recent findings suggest a close relationship between endophyte infection rate in grasses and adverse environment (Hahn *et al.*, 2004; Sugawara *et al.*, 2004). In fact, benefits of evolutionary adaptations to a specific environment in E+ compared to E– grasses may fail completely once host plants are exposed to contrasting conditions (Hesse *et al.*, 2003). For example, E+ plants of perennial ryegrass ecotypes from a dry environment expressed reduced growth under wet conditions, but accelerated regrowth under drought stress when compared to E– plants. Endophyte-infected ecotypes of perennial ryegrass from dry and occasionally flooded environments produced more reproductive stems and seed, but fewer vegetative tillers when compared to E– plants. These responses are common adaptations of plants growing in drought-prone environments. Finally, endophyte infection in ecotypes of



**Figure 1** Incidence of endophyte infection in tall fescue, perennial ryegrass, and meadow fescue across precipitation and temperature gradients in Europe. The annual precipitation increases from south to north, while mean temperature decreases. Modified from Lewis *et al.* (1997) and Lewis (2000b).

perennial ryegrass originating in wet environments expressed heightened sensitivity to drought and reduced regrowth rates following drought. Specific host–endophyte associations may only be beneficial in the site of adaptation and can very likely be antagonistic outside of that environment.

Sleper (1985) proposed that tall fescue ( $2n = 6x = 42$ ) evolved separately north and south of the Mediterranean Sea, as suggested by a lack of cross-compatibility between ecotypes collected in the two regions (Hunt and Sleper, 1981). DNA sequence analysis and fungal endophyte surveys helped identify the ranges of the two tall fescue groups: North Africa to the Western Mediterranean, Iberia and Italy, bounded at the Pyrenees and the Alps (the southern ecotypes), and northern Europe into Iberia, Morocco and Italy (the northern ecotypes) (Craven *et al.*, 2005). Some of the southern ecotypes of tall fescue (from southern Spain, Algeria, and Sardinia) harbor endophytes (designated FaTG-2 and FaTG-3) that are genetically, biochemically, and morphologically different from *N. coenophialum* (Christensen *et al.*, 1993; Tsai *et al.*, 1994; Clement *et al.*, 2001; Piano *et al.*, 2005), whereas *N. coenophialum* is consistently found in northern tall fescue and in those tall fescue lineages introduced from northern Europe into North America, Australia and New Zealand (Craven *et al.*, 2005). Interestingly, many recently developed cultivars of Mediterranean tall fescue (also referred to as summer-dormant types), such as Fraydo (Reed *et al.*, 2004), Prosper (Heritage Seeds, 2005) or Flecha (Bonarino J, Gentos SA, Argentina, personal communication), do not harbor any endophytes suggesting their parental lines were also endophyte-free. Moreover, artificial infection of Mediterranean-origin tall fescue with *N. coenophialum* does not seem to confer any benefits in terms of increased drought tolerance or persistence in hot and dry environments (Malinowski *et al.*, 2005a; Malinowski, unpubl. data, but see Assuero *et al.*, 2000). Thus, extended range of adaptation of tall fescue to drier and hotter environments, as a result of the association with *N. coenophialum*, may be important for the northern ecotypes but not plant ecotypes originating in hot and dry environments.

## Conquering hot and dry environments

If the hypothesis that *Neotyphodium* spp. endophytes primarily enabled cool-season grasses to colonize drier and hotter environments is true, then the ability to survive drought should be commonly expressed by all cool-season grass hosts harboring these endophytes. This would most likely occur by developing efficient mechanisms improving water uptake, conserving water in plant tissues, or protecting meristematic tissues from oxidative stress. Earlier research identified positive effects of the shoot-located *Neotyphodium* spp. endophytes on a range of characteristics increasing drought stress tolerance in the three major agronomic grasses: perennial ryegrass, tall fescue, and meadow fescue, and negligible or detrimental

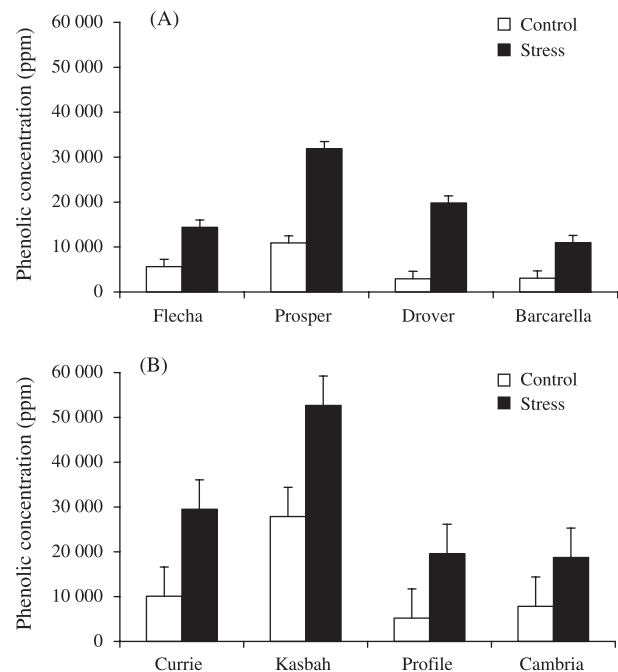
effects at optimal soil water supply (Malinowski and Belesky, 2000). In effect, the proportion of E+ grasses rapidly increases in pastures within a few years (Clay, 1988; Hill *et al.*, 1998). In typically semidry environments, native grass populations also express a very high percentage of E+ plants (Sabzalian *et al.*, 2005). Some recent work proposes, although strictly *hypothetically*, the ecological significance of newly discovered associations of *Neotyphodium* spp. endophytes with nonagronomic cool-season grasses in terms of greater abiotic stress tolerance (Marshall *et al.*, 1999; Vinton *et al.*, 2001). Detailed research on ecology of E+ native grasses, however, is extremely scarce. Nan and Li (2001) conducted a survey of native grasses in China, reporting endophyte infection in 25 species of 13 genera. The authors observed significant increase in root and shoot DM, and tiller number in response to endophyte infection in *Hordeum bogdanii* (Wilensky) and *Elymus cylindricus* (Franch.). In Arizona fescue, a grass native to the south-western USA, endophyte infection increased plant fitness *only* if exposed to soil water deficit (Morse *et al.*, 2002), similar to results obtained with grasses having agronomic utility. Under limited soil water conditions, E+ plants of Arizona fescue had greater above-ground biomass and relative growth rate (RGR) than E– plants that correlated with higher net assimilation rates ( $P_n$ ), lower leaf conductance ( $g_l$ ), and greater specific leaf area (production of thinner leaves). Regardless of soil water availability, E+ plants tended to have lower midday  $P_n$  than E– plants, primarily due to stomatal response, rather than biochemical limitations to photosynthesis. The adaptations are very similar to those described in agronomic grasses such as tall fescue and meadow fescue (Malinowski and Belesky, 2000). In contrast, stomatal functions do not seem to be affected by infection with *N. lolii* in perennial ryegrass, and there are either inconsistent or negligible effects for both physiological (Barker *et al.*, 1997; Amalric *et al.*, 1999) and morphological traits (Cheplick *et al.*, 2000; Lewis, 2000a).

One important adaptation of plants to drought is the expression of an extensive root system that allows water uptake from a greater volume of soil. More massive root systems were reported in E+ compared to E– plants of the three agronomic grasses: perennial ryegrass (Latch *et al.*, 1985), tall fescue (De Battista *et al.*, 1990) and meadow fescue (Malinowski *et al.*, 1997b), and a nonagronomic, rangeland species with the common name of sleepygrass (*Achnatherum robustum* [Vasey] Barkworth) (Hayes and Faeth, 2004). Although *Neotyphodium* spp. endophytes have not been found in roots of host grasses, they do affect root morphology and functions. Malinowski *et al.* (1999b) observed smaller root diameter and longer root hairs in hydroponically grown E+ compared to E– genotypes of tall fescue. A similar response was later shown for perennial ryegrass (Zhou *et al.*, 2003). This phenomenon was not observed in soil-grown plants to date; however, smaller root diameter and longer root hairs will increase root surface area

and should improve the water and nutrient uptake efficiency by plants manifesting these characteristics. In Arizona fescue, endophyte infection did not affect root DM or root-shoot ratio, regardless of available soil moisture (Morse *et al.*, 2002). Root morphology was not quantified.

Secondary metabolites of host–endophyte associations could be involved in the osmotic response of host grasses to soil water deficit stress (Lyons *et al.*, 1990; Richardson *et al.*, 1992; Bush *et al.*, 1993; Elbersen and West, 1996). One theory suggests that loline alkaloids are involved in the regulation of drought responses in E+ grasses (Malinowski and Belesky, 2000). Although we now know that loline alkaloids are produced by *Neotyphodium* spp. endophytes from the amino acid proline, an abundant and common stress metabolite of many plants (Blankenship *et al.*, 2005), direct involvement of the pyrrolizidine alkaloids in drought stress responses has not been documented. Another theory suggests that antioxidants are involved in the superior drought survival of E+ grasses, possibly by increasing tolerance of oxidative stress at the cellular level (Malinowski *et al.*, 2005a). Endophyte-infected grasses contain much higher concentrations of phenolic compounds than their E– counterparts (Powell *et al.*, 1994; Malinowski *et al.*, 1998a; Zhou *et al.*, 2003). Phenolics are potent antioxidants and help protect plant cells from oxidative stress during drought (Blokhina *et al.*, 2003). Endophyte-infected plants of tall fescue (Ayad, 1998; Fike *et al.*, 2001) and perennial ryegrass (Anzhi *et al.*, 2004) express greater activity of superoxide dismutase (SOD) than E– plants. Activity of another enzyme associated with oxidative stress, ascorbate peroxidase, was higher in E+ than in E– plants of perennial ryegrass exposed to zinc (Zn) stress, suggesting that *N. lolii*-endophyte modifies plant metabolism by favoring H<sub>2</sub>O<sub>2</sub> scavenging throughout the catalase process (Bonnet *et al.*, 2000). Interestingly, ongoing experiments (Malinowski, unpubl. data) with summer-dormant, Mediterranean cultivars of tall fescue (naturally free of endophytes) and orchardgrass showed greater concentrations of phenolic compounds in tiller bases when compared to summer-active cultivars of these grass species at the beginning of summer, regardless of soil moisture status (Figure 2). Thus, meristem survival during drought may be a function of protection from oxidative stress in summer-dormant cultivars of a grass species. Protection could be amplified by endophytes in summer-active cultivars of the species. Another group of metabolites common to E– summer-dormant (Volaire, 2002; Volaire *et al.*, 2005) and E+ summer-active cool-season grasses (Carson *et al.*, 2004) appears to be dehydrins, which apparently are correlated with the early sensing of soil water deficit and initiation of dormancy processes. The hypothesis implicating dehydrins in drought-stress tolerance of E+ grasses is worthy of further detailed investigation.

*Neotyphodium* spp. endophytes in seeds of agronomically important grasses are susceptible to heat damage especially



**Figure 2** Phenolic concentrations in tiller bases of controlled (90% soil water capacity) and stressed (gradually imposed soil water deficit from 90% to 15% soil water capacity within 30 days) cultivars of naturally endophyte free tall fescue (A) and orchardgrass (B). For tall fescue, cultivars Flecha and Prosper are summer-dormant (Mediterranean), and Drover and Barcarella are summer-active. For orchardgrass, cultivars Currie and Kasbah are summer-dormant, and Profile and Cambria are summer-active. Bars indicate 1 s.e. (Malinowski, unpubl. data).

during long periods of storage (Siegel *et al.*, 1984; Welty *et al.*, 1987). Temperatures above 36°C are common in dry environments of southern Europe or northern Africa during summer. Hume and Barker (2005) reviewed data on endophyte survival in buried seed that were in contrast to findings on endophyte viability in storage systems. Interestingly, endophyte viability in seeds was higher in drier soil conditions. In the hot and dry environments of Tunisia, Morocco and Italy, endophyte infection frequencies in seeds of some indigenous tall fescue ecotypes were surprisingly high (Clement *et al.*, 2001). We are not aware of any study specifically designed to evaluate endophyte survival rates in buried seeds under natural hot and dry environments; however, there seems to be no apparent problems with vertical transmission of endophytes.

## Soil-root interface and soil microbial processes

Plants developed numerous adaptive strategies to deal with mineral imbalances, ranging from mineral deficiency to excess (Marschner, 1995). Such adaptations may enable certain

species to dominate natural plant communities, especially where mineral imbalances occur. Traditionally, the ecological importance of grass endophytes was discussed in terms of drought tolerance and protection from herbivory. Recently, significant effort was devoted to understand interactions between leaf-located *Neotyphodium* spp. endophytes and root functions of cool-season grasses (Malinowski and Belesky, 2000). Mineral nutrients such as nitrogen (N) and phosphorus (P) were found to be associated with alkaloid production in E+ grasses (Belesky *et al.*, 1988; Arachevaleta, 1992; Azevedo *et al.*, 1993). One important mechanism of N assimilation discovered in E+ grasses was greater activity of glutamine synthetase, an enzyme responsible for  $\text{NH}_4^+$  reassimilation (Lyons *et al.*, 1990). This mechanism helped explain the efficient use of N by E+ tall fescue plants (Arachevaleta *et al.*, 1989). A milestone step in our understanding of endophyte involvement in mineral nutrition was the discovery of chemical modifications in the rhizosphere of tall fescue and the regulatory effects root exudates had on uptake of certain minerals (Malinowski *et al.*, 1998a; Malinowski and Belesky, 1999a, 1999b; Malinowski *et al.*, 2000; Malinowski, *et al.*, 2004). Release of phenolic-like compounds with iron ( $\text{Fe}^{3+}$ ) reducing and aluminum (Al) and copper (Cu) chelating activities facilitates P acquisition by E+ tall fescue plants (Malinowski and Belesky, 2000). Although roots of E+ perennial ryegrass plants also release phenolic compounds (Zhou *et al.*, 2003), not much is known about their involvement in mineral uptake (Malinowski *et al.*, 2004). Rahman and Saiga (2005) evaluated growth of E+ and E- clones selected from native populations of tall fescue in Japan cultivated on Black Andisol (low P content, high contents of other nutrients) and Red Andisol (high P content, low contents of other nutrients) under controlled growth conditions. When grown in Black Andisol, E+ plants had higher cumulative shoot dry weight and daily regrowth rates than E- plants, while in Red Andisol the reverse was true. In the P-deficient Black Andisol, endophyte infection increased P, potassium (K), calcium (Ca) and magnesium (Mg) uptake and transport within plants. Although root exudates were not evaluated in this study, results support the theory of an internal signaling system among the endophyte, grass host, and environment that leads to faster and more accurate responses to environmental factors than those occurring in E- plants.

A few recent observations (Monnet *et al.*, 2001, 2005) suggest greater tolerance of E+ compared to E- plants of perennial ryegrass to Zn. The response was measured in terms of damage to PSII system. The E+ plants were able to maintain photosynthesis and growth functions at higher Zn levels than were E- plants. The chelating abilities of phenolic compounds may be another mechanism enabling symbiotic grasses to cope with phytotoxic metals.

Soil microbial communities are linked with the plant community, the interaction of which may influence biodiversity

and ecosystem functioning (van der Heijden *et al.*, 1998). Emerging studies show that endophytes have some influence on soil microbial processes, suggesting that E+ grasses not only have adaptative advantages in adverse environments, but can modify soil environments to ensure some competitive advantages (Matthews and Clay, 2001). Franzluebbers *et al.* (1999) demonstrated that tall fescue pastures containing a high percentage of E+ plants had greater soil organic C and N concentrations and lower soil microbial activity than pastures with low occurrence of endophyte infection. One explanation might involve endophyte metabolites, such as alkaloids and phenolics produced by the endophyte-grass host association, either actively released into the rhizosphere (Malinowski *et al.*, 1998a, 1998b) or passively released from dead plant material (Franzluebbers *et al.*, 1999). Support for the hypothesis of reduced microbial activity in soils grown with E+ Italian ryegrass was presented by Omacini *et al.* (2004), where leaf litter from E+ Italian ryegrass decomposed more slowly than E- leaf litter.

Grasses commonly form symbiotic associations with mycorrhizal fungi that increase grass host tolerance to drought and mineral deficiency stress (Miller, 1987; Smith and Read, 1997). *Neotyphodium* spp. endophytes can suppress mycorrhizal fungi colonization of grass roots in tall fescue (Baker, 1987; Chu-Chou *et al.*, 1992; Guo *et al.*, 1992), perennial ryegrass (Müller, 2003) and annual ryegrass (Omacini *et al.*, 2005), suggesting alternative strategies to ensure plant survival under suboptimal environmental conditions. The ecological impact of this antagonistic interaction for E+ grasses is not clear; however, as shown by Vicari *et al.* (2002) mycorrhizae may actually reduce insect resistance in E+ perennial ryegrass plants, thus affecting competitive ability against other plants in mixed-sward grasslands. The *in vitro* growth of several pathogenic fungi (i.e. *Fusarium culmorum*, *Dreschlera dictyoides*) was inhibited in the presence of *N. lolii* (Holzmann-Wirth *et al.*, 2001); however, responsible inhibitors were not yet identified.

## Competitive ability and the avoidance of herbivory

The occurrence of E+ grasses in grasslands and managed pastures has had a significant and varied role in grazing animal behavior and human events through time (White *et al.*, 2003). An array of bioprotective alkaloids produced by E+ grass hosts contributes directly to the competitive ability of the associations under a range of growing and management conditions (Bush *et al.*, 1997). Knowledge of interactions of endophytes with agronomic species, and more recently grasses in natural or unmanaged environments, continues to expand (Clay, 1998; Clay and Holah, 1999; Brem and Leuchtman, 2002; Faeth *et al.*, 2004). In general, endophyte infection frequencies seem more variable in natural compared

to managed ecosystems supporting domesticated grasses such as tall fescue and perennial ryegrass. Variability suggests that expression and benefits attributable to endophyte infection depend on the resource environment in which the host–endophyte complex was formed and where it now occurs. Abundant nutrient supply might obscure endophyte advantages, measured in terms of plant growth, in competitive situations, but in some cases appears to facilitate secondary metabolite production and confer herbivory avoidance. Effects of endophyte infection on grazing livestock include depressed voluntary intake (Howard *et al.*, 1992; Parish *et al.*, 2003), altered duration, timing and intensity of grazing events (Eerens *et al.*, 1998; Seman *et al.*, 1999), reduced endocrine function and metabolic homeostasis (Porter and Thompson 1992; Bouton *et al.*, 2002) decreased productivity measured in terms of live weight gain (Hill *et al.*, 2002; Nihsen *et al.*, 2004) and milk production (Thompson *et al.*, 2001), and impaired reproductive processes including reduced conception, increased spontaneous abortions, altered gestation interval duration in females, and reduced spermatogenesis, testicular growth, testosterone production, and libido in males (Porter and Thompson, 1992). The effects on reproduction and ultimately numbers of grazers could contribute to reduced grazing pressure on natural grassland ecosystems with substantial E+ plant populations, and minimize usefulness of infected plant resources in managed agricultural applications.

Grazer selectivity could increase the likelihood that E+ grasses would be consumed and in effect, shift composition and nutritive value of the sward to one that is inhospitable to herbivores because of the greater proportion of E+ plants. While depressed live weight gain arising from altered grazing behavior confounds the utility of E+ tall fescue in pasture, endophyte does help perpetuate swards with substantial E+ grass components, providing some level of primary production (e.g. available herbage in pasture), while at the same time minimizing soil erosion. Mitigation of soil erosion and the transition from intensive cropping to pasture production systems in the south-eastern USA was a major factor in the widespread use of E+ Kentucky 31 tall fescue. The importance and impact of endophyte infection on plant persistence and livestock productivity was unknown when Kentucky 31 was being introduced and applied as soil conservation and plant cover resource. The cultivar was able to grow and persist in pastures in the south-eastern US where other cool-season forage grasses could not. Population selection pressures exerted by large grazing animals, ruminants in the case of E+ tall fescue and perennial ryegrass, occur simultaneously with pressures associated with insects and microbes, including plant pathogens and host genotype. We present observations on influences of endophyte infection on plant responses at the individual and pasture community scale, where resource patches and sward structure dictate herbage productivity and nutritive value.

Grazing livestock performance on pasture in the USA tend to be problematic in the southern and western portions of the zone of tall fescue adaptation, because heat and drought stress influence the host–endophyte association as well as the grazer. Certain manifestations of the tall fescue toxicosis syndrome are weather-related with some aspects occurring in summer when grazer numbers on pasture typically are high and environmental conditions stress grazers. Elevated air temperatures reduced feed intake by cattle grazing E+ tall fescue, especially where temperatures exceeded 31°C (Hemken *et al.*, 1984). Reduced herbage intake was caused by impaired ability to dissipate heat because of distal vasoconstriction (Rhodes *et al.*, 1991). High temperature and restricted water availability increased alkaloid accumulation in E+ tall fescue plants (West, 1994), so that increased concentrations of bioactive alkaloids compounded the effects of extreme weather conditions on the grazer. Manifestations of the toxicosis syndrome, generally termed ‘fescue foot’ associated with distal vasoconstriction, can occur when cattle consume stockpiled E+ tall fescue in colder periods of the year. Alkaloid-induced physiological responses apparently increase grazer sensitivity to environmental extremes.

It seems that abiotic factors associated with regional and local climate, influence the host–endophyte–grazer complex, and can do so at many levels. For example, interactions could occur as:

- Host–endophyte genotype (plant and fungal genome expression when growing as symbionts with expression ranging from mutualism to antagonism; components could include native and novel nonergogenic endophytes, and high carbohydrate plant hosts)
- Host–endophyte genotype  $\times$  soil resources (mineral nutrients, pH, water)
- Host–endophyte genotype  $\times$  grazer genotype (breed and within breed variation; e.g. *Bos taurus* compared to *B. indicus*)
- Host–endophyte genotype  $\times$  grazer genotype  $\times$  abiotic environment (soil physical and biochemical limitations and climate associated with extremes in the range of plant adaptation)

All of the interactions are likely to be affected by extrinsic factors associated with management of agroecosystems and might not always be expressed in the same manner or to the same degree. For example, environmental conditions may stimulate plant host growth exceeding that of the endophyte within. This is a fairly typical response because the endophyte, being noninvasive, requires the intercellular space in which to grow and logically then plant growth should precede that of the endophytic mutualist.

There seems to be some difference of opinion regarding the nature of endophyte infection depending on whether the host plants are part of managed agroecosystems, or range and native grasslands. Endophyte associations with Arizona fescue are considered parasitic (Faeth and Sullivan, 2003), whereas others (Saikkonen *et al.*, 1998; Spyreas *et al.*, 2001)

suggest that a continuum of expression occurs between the symbionts ranging from parasitic to mutualistic. In their experiments, endophyte infection of tall fescue in intensively defoliated plots was associated with increased sward diversity, which they attributed to increased drought tolerance of E+ plants. It could be that mowing favored the infected plants that had accelerated leaf extension thereby increasing as a sward component through resource consolidation and competitive ability along the lines hypothesized by Barthram *et al.* (2005). In Barthram's experiments, resistance to invasive neighbors was related to residual sward surface height and the extension of new or existing leaves into space (to capture light). Because sward residue height was inversely related to resistance to invasion, the increased species richness observed by Spyreas *et al.* (2001) could be a product of leaf expression as well as increased water stress tolerance. In the case of tall fescue, sward residue height was directly related to alkaloid concentrations (Belesky and Hill, 1997) with probable impacts on alkaloid content and resistance to herbivory. Leaf elongation depended on host genotype especially early on during seedling growth (Belesky *et al.*, 1989). In some instances, E- plants continue to grow in the presence of abiotic stresses (for instance water deficit), whereas E+ plants could adjust to stress by minimizing exposure to that particular stress (leaf rolling or slower growth compared to E- plants) and avoid physical and physiological damage.

Diverse communities seem to resist encroachment by other species especially where distribution of species is uniform rather than patchy (Tracy *et al.*, 2004). Mutualistic endophytes such as those in tall fescue enhanced ability of the association to invade established plant communities, regardless of species composition of that community, although this is not always the case. Tracy and Renne (2005) observed that mixed species swards including an E- tall fescue cultivar, used to renovate a well-established tall fescue pasture, had few E- plants after 3 years. Shifts in sward composition could be a function of vigorous competition by E+ plants or preferential selection by grazers of plants other than E+ tall fescue. Rudgers *et al.* (2005) suggest that host-fungal associations may be more important early in the establishment phase than during the later growth phase of the sward. Changes in E+ grass litter and effects on mineral nutrient pools could influence the floristic composition of the sward and herbage productivity. Omacini *et al.* (2005) present an excellent synthesis of host-endophyte associations and ecosystem processes. A hierarchical relationship occurs at differing spatial and temporal scales. At the individual level, the endophyte influences individual host traits, affecting nutrient acquisition and interaction with other individuals in the sward and organisms at other trophic levels. This could influence structure of the sward (neighborhood) where abiotic stresses have some impact on productivity and persistence. At the community level, interaction with other species and resource distribution

patches occur. Endophyte infection affects nutrient pools, food web structure and ability to resist invasiveness, enabling E+ grasses to be more competitive in mixed species situations (Malinowski *et al.* 1999a).

A model (Faeth and Fagan, 2002) of the interaction of endophyte infection and stresses, predicts that low alkaloid-producing endophyte-grass associations should persist in population when soil nutrients and herbivory are low. Alternatively, high alkaloid endophyte-grass associations are favored under increasing herbivory and increasing soil nitrogen, at least to some point. At very high soil nitrogen levels, E- plants may be favored over either alkaloid-profile type of E+ plants. Predictions are supported by patterns of infection and alkaloid production in nature, and in field experiments; however, plant genotype and environmental factors, such as available water, interact with endophyte to influence host plant performance. The benefit of endophyte infection is less when nutrient and water availability is high, corroborating observations that endophyte facilitates host growth in stressful environments.

A host-endophyte association adapted to and arising from the environmental selection pressures of extreme conditions (e.g. high temperature and periodic and intense soil water deficits) and capable of producing alkaloids, might not express the same competitive fitness nor elicit the symptoms of fescue toxicosis in grazers when grown or used in a less stressful environment. Expression of toxicosis depends on the environment in which the association is grown, the nature of that association and management (e.g. livestock breed, nutrient inputs, grazing scheme) factors that influence the allocation and use of resources by the host-endophyte complex. Alkaloids accumulate under stressful conditions such as drought or when intermediate compounds, including numerous polyamines that can ameliorate the physiological impacts of water stress or serve as precursors to alkaloid synthesis, accumulate when plant growth is slow.

## Domestication of *Neotyphodium*-endophytes

Recent shift from quantitative to qualitative assessment of mechanisms regulating adaptation of *Neotyphodium*-grass associations to abiotic stresses has resulted in domestication of the endophytes, that is, selection of novel endophyte strains that produce minimal amounts or no alkaloids detrimental to grazing livestock, yet appear to retain host tolerance to abiotic and biotic stresses (Tapper and Latch, 1999; Bouton *et al.*, 2002; West and Gunter, 2004). The major emphasis in selecting novel endophytes in the USA, Australia and New Zealand has been safety to grazing animals. Results from grazing experiments on Jesup tall fescue reinfected with MaxQ endophyte AR542 (Macon *et al.*, 2004) and HiMag tall fescue reinfected with endophyte UA4 (Nihsen *et al.*,



2004), and perennial ryegrass reinfected with the Plus AR1 (New Zealand Agriseeds, 2005) or Plus Nea2 (Heritage Seeds, 2003) endophytes confirm excellent animal performance.

Ecological consequences of novel endophyte introduction into agroecosystems are not well understood. Research is currently underway to evaluate the persistence of grasses infected with novel endophytes in drought-prone environments. Bouton *et al.* (2002) observed about 15% decline in stand survival of summer-active Jesup MaxQ tall fescue when compared with Jesup infected with a wild-type endophyte. Both accessions survived much better than Jesup E– from 1997 through 2002 in the south-eastern USA. Tiller survival of Grasslands Flecha, a summer-dormant type tall fescue, infected with MaxQ endophyte AR542 was greater than that of E– plants during summer drought in the second and third growing seasons in a semiarid environment in North Texas, USA. Endophyte infection had no effect on tiller survival during an extreme summer drought at the same location in 2003 (Malinowski *et al.*, 2005b). In the same study, neither AR542 nor native endophyte strains contributed to the persistence of summer-active tall fescue cultivars Jesup and Georgia 5 that did not survive the first summer drought. West and Gunter (2004) reported persistence of HiMag tall fescue infected with novel endophytes comparable with E+ Kentucky 31 and enhanced persistence relative to E– HiMag after 5 years of field trials in south-west Arkansas. Preliminary data (Malinowski, unpubl. data) do not confirm any advantages of the AR1 strain of *N. lolii* endophyte for drought tolerance in Aries and Quartet perennial ryegrass grown in a semiarid environment in North Texas, USA. In terms of mineral stress tolerance, the novel *N. coenophialum* strain, AR542, elicits similar responses in tall fescue host plants to those shown for the wild-type endophyte strains (Malinowski *et al.*, 2004), i.e. exudation of phenolic-like compounds with metal chelating activity. Basic studies by Belesky *et al.* (2004) and Belesky and Burner (2004) suggest a slight disadvantage to juvenile plants of Jesup tall fescue of harboring the novel endophyte strain AR542 in terms of reduced germination and tillering rates when compared with plants infected with a native endophyte strain, or E– plants. Grasses infected with novel endophytes may experience a competitive disadvantage once exposed to stresses associated with defoliation and variation in growing conditions. Preliminary results, however, do not confirm any differences in terms of persistence of grasses that are endophyte free, or infected with either native or novel endophytes (Barker *et al.*, 2005; Hume and Barker, 2005). One may argue that the time span in those studies was too short to make a comprehensive statement about plant persistence. For example, biotic stresses like insect herbivory (Popay and Baltus, 2001; Popay *et al.*, 2004; Hunt and Newman, 2005) or nematode infestation (Timper and Bouton, 2004) were detrimental to grasses infected with novel endophytes when compared to grasses infected with native endophyte strains. Hunt and

Newman (2005) suggest that AR542 infection does not provide the same degree of protection from certain insects (e.g. aphids) as does infection with the common strain endophyte. Repeated short-term disturbances of competitive ability might have drastic long-term consequences for stability of novel associations in managed agroecosystems. If similar results are found for other invertebrate herbivores, then the use of novel endophyte associations may be less useful in the wider sense than previously thought. The widespread conversion of tall fescue pastures, currently infected with common endophyte, to pastures composed of cultivars bearing the novel AR542 strain could lead to a general increase in the prevalence of certain insects and, potentially, diseases they spread. However, such conversion might be beneficial to native invertebrate herbivores, but not necessarily the host species.

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